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CRDEC-TR-88142

MUSTARD CONTACT HAZARD, CORRELATION OF EFFECTS IN SKIN WITH CONTAMINATION LEVELS RECOVERED FROM DENTAL DAM AND PAINTED STEEL SURFACES I. ANIMAL AND CHEMICAL DATA

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August 1988

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Testing was performed to determine if the degree of skin irritation in rabbits resulting from direct or vapor contact with mustard (HD) could be used to predict the amount of HD that had caused the injury. A series of tests were conducted to determine the degree of skin injury from known levels of HD contamination. In a second series of tests, skin injury was used to quantify the level of HD contamination. These results indicate that skin injury is related to the total amount of HD in contact with the skin, but only when the skin was contacted by a discrete droplet. If the HD contamination resulted from a source other than a discrete droplet (i.e., residue in a painted surface), then the correlation between agent dose and skin irritation cannot be closely predicted. It was also determined that rabbit skin is much more sensitive to the insult of HD than swine skin. Rabbit skin was about five times more sensitive at the lower levels of contamination (continued on reverse)

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18. SUBJECT TERMS (continued)

Polyurethane Painted Steel
Isopropanol Rinse
Skin Irritation
Erythema
Edema
Eschar Formation
Diethyl Phthalate (DEP)
Pilot Study
Research Study

19. ABSTRACT (continued)

(below 1.0 mg). Therefore, if a laboratory animal indicator of HD surface contamination is needed, the rabbit would be the species of choice.

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PREFACE

The work described in this report was authorized under Project No. 1L162706A553(B), CB Defense and General Investigation, Technical Area 3-D, Individual Protection. This work was started in February 1985 and completed in November 1985. The experimental data are contained in laboratory notebook no. 85-0007.

In conducting the research described in this report the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. These investigations were also performed in accordance with requirements of AR 70-18, Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs, and approved by the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Chemical Research, Development and Engineering Center (CRDEC) (Pilot Study Number 21085000B183 approved 28 June 1985 and Research Protocol Number 21085000A184 approved 26 July 1985.)

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MUSTARD CONTACT HAZARD, CORRELATION OF EFFECTS IN SKIN WITH CONTAMINATION LEVELS RECOVERED FROM DENTAL DAM AND PAINTED STEEL SURFACES
I. ANIMAL AND CHEMICAL DATA

1. INTRODUCTION

Three recently completed studies were designed to explore the direct and vapor contact hazard of mustard (HD), thickened mustard (THD), VX, and thickened VX (TVX) using rabbits as biological models.[1,2,3] Visible skin irritation[1,3] or toxic signs and red blood cell (RBC) cholinesterase (ChE) depression were indicators of a contact hazard.[2]

Klein[4] defines contact hazard as: "Given a surface that has been contaminated with a liquid chemical agent and that surface undergoes a process after which the agent no longer can be detected as a liquid, contact hazard is that situation in which a toxicological hazard can result if an individual then touches that surface with bare skin." Previous investigations[4,5] indicate that surfaces appearing clean do, in fact, contain contamination capable of causing a physiological response in a biological target (i.e, an individual or an animal).

Two models that describe the method of transfer of contamination from its source to the contacting surface have been proposed. Sidman and his co-workers at Arthur D. Little Company (Cambridge, MA) have proposed an absorption model predicated on the assumption that agent desorbs from a surface in the vapor phase and is sorbed by the contacting surface in turn.[6] A second model, proposed by Klein (unpublished data), assumed that partitioning the agent between the contaminated surface and the contacting layer may contribute to the transfer process. In this model, it is postulated that the contaminated surface can be compared to a pseudoliquid, and the agent transfers across the interface to the contacting layer (as between two immiscible liquids in contact) at a rate higher than that for a vapor transfer.

Tests conducted earlier[1,2,3] have shown that agent does transfer as both a vapor and a partitioning phase (pseudo-liquid). Given identical contamination/contact circumstances, damage from direct contact is more severe than damage from vapor contact. These previous studies were designed to test the two proposed models as well as determine whether a measurable (skin damage) response could be evaluated in the rabbit.

The present study, designed in several phases, will determine if a measured amount of agent contamination could repeatedly damage (by measurement) skin and if skin damage could

be used as a determining measurement to predict the amount of agent causing the damage.

In the control phase of this project, we determined the accuracy of our agent delivery systems and analytical procedures. During the second phase, skin irritation was studied in rabbits and swine following exposure to known levels of agent contamination. The third phase involved measuring skin damage in rabbits from unknown HD contamination levels desorbing from painted steel surfaces. The painted surfaces were alkyd and polyurethane as applied and aged on steel. Additional information on agent transfer was garnered in Phase II by using dental dam as an absorptive layer. In all phases, a complete chemical analysis of all contaminated surfaces was conducted, and damage to rabbit and swine skin was photographed.

Rabbits were used in this study because this species is the standard animal used when conducting skin irritation studies. Swine were used to be more predictive of damage to human skin and as one of five species involved during multi-species toxicity studies.[7,*] Therefore, we are equipped to handle this species in a toxic environment such as the one involved when testing HD. Other investigators have used swine as an animal model in comparison to human skin,[8,9] and Bronaugh[10] states that pig skin often gives permeability values similar to those for human skin. Because of limited resources, rabbits were used exclusively in Phase III. This report will summarize the animal and chemical data obtained during the study. A second report (Part II) will provide photographic documentation of rabbit and swine skin irritations observed in these tests.

2. MATERIALS

2.1 Chemical Agent.

Distilled mustard (HD) with a purity of 97.9 to 98.7% by NMR was used in these tests. HD has a density of 1.27 g/mL at 25 °C, and all tests were conducted with dosages corrected to volume based on this density. This HD sample was identified on receipt number 1-X-DRSMC-CLB-CO-6 and was obtained from T. Blades, EA Chemical Agent Storage Yard (CASY). During the study, the sample was not refrigerated and maintained a clear color.

2.2 Animals.

2.2.1 Rabbits.

Three hundred twenty-four adult, New Zealand, White Rabbits (sex as available) were used in this study. They were

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*Part of information was provided by Manthei et al., Research Directorate, CRDEC, unpublished data.

requested in a weight range of 2.3 to 3.0 kg and were obtained commercially by the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) Animal Resources Branch (Aberdeen Proving Ground, MD). Rabbits were quarantined for approximately 7 days prior to use, were maintained on approved rabbit chow, and had food and water available ad libitum. Housing was in single unit, stainless steel cages with temperature and humidity maintained at 75 ± 5 °F and 40-60%, respectively. Lighting was on a 12-hr light/dark cycle.

In addition to the 324 rabbits used, 10 additional rabbits were ordered as backups in case the skin of a rabbit was not suitable for dermatologic work. At the end of the experiment, the 324 rabbits were euthanized by an intravenous (ear vein) injection of T-61 (euthanasia solution) and incinerated. The unused rabbits were transferred to USAMRICD and assigned to another investigator.

2.2.2 Swine.

Eighteen young, male, intact (noncastrated), Chester White/Yorkshire Cross Swine weighing 10-15 kg were obtained commercially by the USAMRICD Animal Resources Branch and quarantined for 7 days prior to use. They were maintained on commercial hog chow and had food and water available ad libitum. The swine were housed individually in vinyl-coated swine pens with temperature and humidity maintained at 75 ± 5 °F and 40-60%, respectively. On test days, they were placed into chemical fume hoods, exposed for 24 hr, and returned to the swine pens. After the 72-hr test, the swine were euthanized by an intravenous (ear vein) injection of T-61 and incinerated.

2.3 Dental Dam.

The dental dam used in these experiments was tan colored, unflavored, pure latex, extra heavy #00536 manufactured by Hygenic Corporation (Ackron, OH). It was cut into 1-in. by 2-in. strips for test purposes and was kept refrigerated prior to use.

2.4 Steel Test Plates.

The steel test plates used were 1-in. by 2-in. long and cut from painted stock plates measuring 4-in. by 12-in. by 0.040-in. thick. These plates were primed with epoxy primer MIL-P-52192B and were finished with the following paints:

- Polyurethane Forest Green Paint
  MIL-C-46168
  #62441 Randolph Products
  Thinner, Polyurethane MIL-T-81772
  Film thickness of 3.0± mils

- Alkyd enamel, Forest Green Paint
  MIL-E-52798A
  Film thickness of 3.0± mils

The painted plates were aged at room temperature and conditioned for 1 year prior to the plate-cutting process. As in earlier tests, neither paint was exposed to direct sunlight.[1,2] According to military specifications, paint film thickness required was 2.7-3.3 mils.

2.5 Stainless-Steel Templates.

When conducting vapor contact tests, stainless-steel templates[1,2] were used to hold the painted steel plates 1 cm from the skin of the test animals. These templates had a recessed lip that supported the test plate and a 1/4-in. rim at the base that provided a border for taping the apparatus to the animals' clipped skin. By sealing the template to the skin, a closed cell was created, preventing HD vapors from escaping the test site.

2.6 Syringes and Syringe Adaptor.

In these experiments, three sizes of syringes were used to deliver micogram and milligram amounts of neat HD. All syringes were carefully calibrated with water, and the delivery was then calculated for HD by correcting for its density, 1.27 g/mL.

- A #705 Hamilton 50-µL syringe delivered HD at 98.43 divisions (div)/mg.

- A #X8637 0.25-mL syringe delivered HD at 8.998 div/mg.

- A #UV191 1.0-mL syringe delivered HD at 5.995 div/mg.

An Agla micrometer syringe holder was used to aid the accurate delivery of these small amounts of agent. This micrometer-driven device provided an additional accuracy of 50 div/revolution. The micrometer device was manufactured by Shardlow Micrometers, Limited (Sheffield, England). The accompanying syringe holder was manufactured by Burroughs Welcome and Company (England).

2.7 Solvents and Reagents.

- Isopropyl Alcohol
  - FSN-6505-00-299-8095
    Isopropyl Alcohol, USP, 12/83
    DLA 120-84-C-0658
    Batch: 83112
    Phipps Products Corporation
    Boston, MA

MCB (Isopropanol) Reagent, A.C.S., PX 1835
MCB Manufacturing Chemists, Incorporated
Associate of E. Merck, Darmstadt, Germany
Cincinnati, OH

- Diethyl Phthalate, DEP
  $C_{12}H_{14}O_4$
  F.W. 222.24, purified
  Lot 745483
  Fisher Scientific Company
  Silver Spring, MD

3. METHODS AND RESULTS

3.1 Animal Preparation and Handling.

3.1.1 Rabbit Pilot Study.

During the pilot study, rabbits were identified by metal ear tags and a black ink ear number (1-36). Approximately 18 hr prior to testing, the animals were clipped free of dorsal hair. On test days, each rabbit's exposure site was carefully demarcated in black ink so it could be identified for the next 72 hr. Rabbits were placed into neck collaring stanchions and chemical-filtered fume hoods with a face velocity of 150 lfpm $^{+}$30. Contact with HD was for 60 min, then the contaminated area was gently wiped with isopropanol to remove any residual-free HD. The rabbits remained in the hoods for an additional 23 hr. They were observed for skin irritation and were evaluated according to procedures in the Code of Federal Regulations (16 CFR 1500.41)[11] and according to the Draize technique.[12] Skin was evaluated at 24-, 48-, and 72-hr postexposure and photographed each time if irritation was present. If the irritation had reversed to normal, a post recovery photograph was taken.

Rabbits were tested in groups of three during the pilot study. During the vapor test phase, the agent was applied (as discrete single droplets) either to bare skin or to steel test plates (unpainted side).

3.1.2 Rabbit Research Study.

During the research study, rabbits were tested in groups of eight. This increase in the number of animals per test group was to increase statistical confidence. All methods of testing, skin evaluations, and photography were identical to procedures used during the pilot study. However, in this study, there was no liquid HD applied to rabbit skin. All test plates had been contaminated and isopropanol rinsed prior to skin contact. The only HD present was trapped either in or under the paint surface.

3.1.3 Swine.

Swine were tested during the pilot study to develop a relationship of rabbit/swine response to HD as applied to bare clipped skin. Eighteen swine, in groups of three, were prepared for testing by gently clipping their dorsal hair 18 hr prior to testing. The animals were placed into fume hoods with a face velocity of 150 lfpm ±30. Each swine was large enough (10-15 kg) to allow for both a direct and vapor contact dose. Swine were restrained in cloth slings and were secured with leg ties. The exposed skin was placed directly into the air flow, and as with the rabbits, the exposed skin was wiped with isopropanol 60 min after contact started to remove any free residual HD. At 24 hr, swine were removed from their restraints, photographed, and returned to their individual holding pens. Photographs were also taken of each exposure site at 48 and 72 hr. Skin irritation was evaluated using the same procedure for rabbits.

3.2 Skin Irritation Evaluations/Photography.

Skin irritation in rabbits and swine were evaluated according to procedures outlined in the Code of Federal Regulations (16 CFR 1500.41)[11] and the Draize technique.[12] Observations of skin damage were made at 1, 24, 48, and 72 hr after agent contact. Scoring of skin irritation was done according to procedures listed in Table 1. The final irritation score represented the average score from three rabbits or swine during the pilot study and eight rabbits during the research study. For purposes of irritation ratings evaluation, only the 24- and 72-hr readings were used. The size of all observable irritations was measured to the nearest 0.125 (1/8) in., and the area of damage for each eschar formation (or necrosis and blanching), erythema, and edema were recorded and photographed for each animal. For the purpose of comparison among dose levels and test conditions, the damage was rated as follows: a final irritation score of 5.0 or greater was considered to be a primary skin irritant; 2.0 to 4.99, a moderate skin irritant; 0.01 to 1.99, a mild skin irritant; and 0.00 was considered as nonirritating to either rabbits or swine. As stated, this irritation was based on observable skin damage and did not include any possible minor congestion to vasculature that could not be seen below the skin's surface.

Color photographs were made of all skin damage observed on rabbits and swine. If damage occurred, it was photographed for up to 72 hr or until recovery occurred prior to 72 hr.

Table 1. Procedures for Scoring Skin Irritation.

Skin Reaction	Value*
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in death)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (easily perceptible)	2
Moderate edema (edges of area well defined by definite raising)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

3.3 Control-Calibration Studies and Chemical Analysis Procedures.

To develop precise techniques for the HD delivery to skin and the calibration of analytical procedures, control studies were done with neat HD applied to glass, steel, and dental dam. These samples were then analyzed using the DB_3 test.[13]

Syringes were calibrated to deliver neat HD in microgram or milligram amounts when using a blunted 27-gauge, stainless-steel needle and a micrometer-driven syringe holder (Agla micro-syringe). The first study involved delivering HD in doses ranging from 0.010 to 3.20 mg onto glass cover slips. This test showed that we could not accurately touch off small amounts of HD to glass, especially in the low microgram range. Amounts of HD in the milligram and gram ranges could be rather accurately delivered as analyzed and shown in Table 2. This wide disparity of delivery versus recovery of HD was thought to be related more to the physical touching of the liquid HD onto glass than to the syringe and analysis technique. A second series of tests was done using polyurethane painted steel plates as the agent recipient (Table 3).

Table 2. Precision - Accuracy of HD Applied to a Glass Surface and Analyzed Chemically.

HD Applied mg/plate	HD Recovered/Chemical Analysis		
	Mean ± S.D.* (µg)	Range (µg)	Recovery (%)
0.010	2.38 ± 1.97	0.8 - 6.0	23.80
0.032	8.88 ± 7.56	1.1 - 20.0	27.75
0.100	85.42 ± 18.80	52.5 - 105.0	85.42
0.320	314.58 ± 12.79	305.0 - 337.5	98.31
1.000	977.08 ± 111.36	812.5 - 1162.5	97.71
3.200	3216.50 ± 51.20	3129.0 - 3255.0	100.52

*Average value for six samples at each dose level.

Table 3. Precision - Accuracy of HD Applied to Polyurethane Painted Steel and Analyzed Chemically.

HD Applied mg/plate	HD Recovered/Chemical Analysis		
	Mean ± S.D.* (µg)	Range (µg)	Recovery (%)
0.010	5.10 ± 1.73	4.1 - 7.1	51.0
0.032	17.67 ± 5.69	13.0 - 24.0	55.2
0.100	65.00 ± 28.0	32.5 - 87.5	65.0
0.320	301.83 ± 38.39	257.5 - 324.0	94.32
1.000	925.00 ± 64.95	850.0 - 962.5	92.50
3.200	3138.33 ± 112.51	3010.0 - 3220.0	98.07

*Average value for three samples at each dose level.

Although there were indications of an increase in delivery accuracy at the 0.010- and 0.032-mg doses, the other four dose levels did not show increased accuracy. The three highest doses in the +90% range were considered to be within limits desired when accounting for evaporation while working in a fume hood at 150 lfpm. A third series of doses were then applied to dental dam. For this test, only the doses of 0.010 to 0.100 mg were done. This time the sample size was increased to 12 each. The procedure was to place 1-in. squares of dental

dam on top of a steel plate inside the fume hood. The HD was then applied to the dental dam with a standard gentle touch off, and the dental dam was immediately retrieved with a forceps and placed into 20 mL of diethyl phthalate (DEP). The results of this test looked more promising, especially considering the small amount of volatile liquid for which we were attempting to account. Table 4 lists the results of this test.

Table 4. Precision - Accuracy of HD Applied to Dental Dam and Analyzed Chemically.

HD Applied (mg/dam)	HD Recovered/Chemical Analysis		
	Mean ± S.D.* (µg)	Range (µg)	Recovery (%)
0.010	8.17 ± 2.12	4.2 - 11.8	81.70
0.032	25.48 ± 5.44	17.0 - 36.0	79.65
0.100	97.50 ± 8.12	85.0 - 112.5	97.50

*Average value for three samples at each dose level.

The results of the above three control and accuracy tests indicated that there could be a loss of precision with delivering microgram amounts of neat HD to surfaces such as glass, painted steel, and to some extent, dental dam. However, dose levels in the milligram range could be fairly well controlled. Problem areas to be concerned with during the delivery of such small amounts of a volatile agent include: speed of delivery and recovery, temperature, humidity, hood air flow, angle of touch off, etc. Because each of these factors plays a role in the final analysis, along with accuracy of the analytical procedures, we felt that we could continue the project but would have to accept variations. For use in this study, a series of HD calibration curves were developed by the analytical chemistry personnel. These data are shown in Table 5. Dilute HD standards were made up in DEP and ranged from 0.04 µg/mL to 9.48 µg/mL. Two AutoAnalyzer IIs (AAII) were used during these tests and throughout the research portions of this program. The first AAII was calibrated for 0.04 to 2.7 µg/mL. The second AAII was calibrated for standards between 0.5 and 9.48 µg/mL. Actual test samples were placed into either 20, 50, or 100 mL of DEP depending on the dose of HD applied. With these two AAII systems, all samples could be analyzed. The detection limit for lower concentrations was 0.04 µg/mL/2 chart divisions. This meant that for the 20-mL samples the limit was

0.8 µg/sample; for the 50-mL samples the limit was 2.0 µg/sample; and for the 100-mL samples the limit was 4.0 µg/sample. For very high concentrations, appropriate dilutions were made in DEP. For high sensitivity (low concentrations), the standard calibration on the colorimeter was set at 9.00, while for low sensitivity (high concentrations), the standard calibration was set at 1.00. The concentrations of all samples in the research study fell within the range of these two curves, reducing the error factor in the results.

Table 5. Calibration Curves - HD/DEP - AutoAnalyzer II.

Low Concentrations			High Concentrations		
Standard Numbers	µg/mL	Chart Divisions	Standard Numbers	µg/mL	Chart Division
1	0.07	2.0	I	0.59	5.5
2	0.14	4.0	II	1.19	9.5
3	0.34	8.0	III	2.37	18.0
4	0.68	15.0	IV	4.74	38.0
5	1.36	29.0	V	7.11	52.5
6	2.04	44.0	VI	9.48	67.5
7	2.72	57.5	--	--	--

3.4 Pilot Study.

Following the completion of the control calibration and chemical analyzer verification studies, we began the pilot study using rabbits and swine with neat, discrete HD deposited on skin directly and indirectly by a vapor transfer technique.

The purpose of the pilot study was to develop a dose-response curve for the effects of discrete liquid mustard on rabbit and swine skin following 60-min contact. The six logarithmic doses of HD selected were 0.010, 0.032, 0.100, 0.320, 1.0, and 3.2 mg. As stated, a direct contact and vapor contact test were done.

3.4.1 Direct Contact Phase.

In the direct contact phase, neat HD was deposited as a discrete droplet on clipped rabbit skin (three rabbits each dose) and allowed to contact skin for 60 min. After 60 min, if any free liquid HD remained, the test site was gently blotted with absorbent (soft) tissue to stop the continued exposure.

There were no occlusive coverings used in this test. Swine were also tested in groups of three, and procedures were the same as those for the rabbits. Skin damage was observed as previously described and was photographed.

3.4.2 Vapor Contact Phase.

During the vapor contact phase of the pilot study, additional rabbits in groups of three were used; whereas, the swine used during the direct contact phase were also used in this vapor phase. This was done because swine had such a large skin surface area. The vapor contact test was conducted on one side of the midline and the direct contact test on the other. For vapor contact, the template was taped to the skin and then a steel plate contaminated with the HD droplet was sealed into the template for 1 hr. In this case, the HD was applied to unpainted steel so as not to be influenced by any factor such as paint. The entire test apparatus was sealed with tape and polyethylene film so any HD vapors would be trapped in the test site. After the 60-min exposure, the HD source was removed, and the test site was blotted with tissue. The rest of the study was the same as the one performed for the direct contact phase.

3.4.3 Rabbit Test Results.

Rabbits exposed to HD by direct and vapor contact for 60 min developed skin irritation within this specified time. All 18 rabbits' skin in the direct contact phase was irritated, and the three highest dose levels in the vapor phase (0.320, 1.0, and 3.2 mg) showed irritation as early as 60 min after contact initiation (Table 6). Size of skin damage (square inch) was related to the dose, especially when looking at the direct contact phase. During the vapor phase, dose levels of 0.100 to 3.2 mg produced similar erythema responses as the HD vapors probably completely filled the template (1 in. by 2-in.), and the damage then spread beyond the original exposure site. This spreading effect was also seen to a lesser degree in the skin of the direct contact animals. By examining the Primary Irritation Index (P.I.I.) scores, we see that all dose levels produced primary skin irritation by direct contact (score of 5.0 or greater) with only small increases in damage severity associated with an increase in dose level.

In the vapor phase, a dose of 0.01 mg produced no visible skin damage, and 0.032 mg produced damage in one of three rabbits. The remaining four doses produced moderate-to-severe injury to skin with a slight dose-response relationship. This effect is noted in the P.I.I. scores for the vapor animals where we see scores of 0.00, 0.42, and then scores of 7.21-7.50, indicating no real differences in the intensity of the damage at the higher dose levels.

Table 6. Pilot Study, Skin Irritation in Rabbits Following Direct and Vapor Contact with HD for 60 Minutes.

HD Dose (mg)	Exposure Type	Mean[a] Area of Skin Damage ($in.^2$) (24 hr)			(72 hr)			
		Erythema	Eschar[b]	Edema	Erythema	Eschar	Edema	P.I.I.[c]
0.010	D	0.065	--	0.065	0.038	0.0079	0.038	5.83
0.032	I	0.089	--	0.089	0.035	0.0079	0.035	6.42
0.100	R	0.188	--	0.188	0.091	0.0287	0.091	6.92
0.320	E	0.313	--	0.781	0.229	0.046	0.360	7.84
1.000	C	0.922	--	2.417	1.041	0.206	1.365	7.67
3.200	T	1.479	--	4.44	1.234	0.531	1.89	7.84
0.010		0.00	--	0.00	0.00	0.00	0.00	0.00
0.032	V	0.467	--	0.467	0.25	0.00	0.25	0.42
0.100	A	3.177	--	4.48	2.677	1.04	4.84	7.21
0.320	P	4.604	--	6.19	4.719	2.167	5.79	7.50
1.000	O	3.67	--	8.23	4.81	2.92	5.15	7.25
3.200	R	4.25	--	7.40	6.06	3.09	6.48	7.25

[a]Three rabbits/calculation.
[b]At 24 hr, there was blanched skin on the direct contact phase but no eschar formation.
[c]P.I.I. based on 16 CFR 1500.41 and Draize Technique.

3.4.4 Swine Test Results.

Because we were trying to produce a dose-response relationship for HD-produced irritation, swine were added as a test species during this pilot study. Swine skin is considered to be a model for human skin. The results of the swine tests are shown in Table 7. However, swine appear to be somewhat less sensitive than rabbits to the irritating effects of HD by direct and vapor contact. Figure 1 shows the 24-hr erythema response for rabbits and swine and indicates that swine are less sensitive when the same dose levels of HD are contacted to their skin. Table 8 lists the rabbit/swine erythema ratios at 24 hr. If we disregard the ratio 16.7 at the 0.010 mg dose, the remaining five doses produce a mean ratio of 4.9 ± 0.96 with a range of 3.8-5.7. From this limited response data, we can see that swine are approximately five times less sensitive than rabbits to the local effects of HD when it is in direct contact with clipped skin. It would be expected that higher dose levels (greater than 3.2 mg) would probably reduce the ratio toward 1.0

as seen in the vapor contact ratios where we see ratios of 1.2-3.5. Based on these results, it appears the rabbit is the species of choice if a sensitive responder is wanted to detect possible HD surface contamination.

Table 7. Pilot Study, Skin Irritation in Swine Following Direct and Vapor Contact with HD for 60 Minutes.

HD Dose (mg)	Exposure Type	Mean[a] Area of Skin Damage ($in.^2$) (24 hr)			(72 hr)			
		Erythema	Eschar	Edema	Erythema	Eschar	Edema	P.I.I.[b]
0.010	D	0.0039	0.0013	0.000	0.0039	0.0013	0.00	2.33
0.032	I	0.0156	0.0039	0.0156	0.0156	0.0039	0.00	4.50
0.100	R	0.0353	0.0104	0.0353	0.0156	0.0068	0.0235	5.50
0.320	E	0.0796	0.047	0.0796	0.0678	0.0392	0.0625	5.34
1.000	C	0.1615	0.1094	0.2135	0.2083	0.1719	0.4740	7.84
3.200	T	0.3906	0.3906	0.5833	0.4219	0.4219	0.667	7.34
0.010		0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.032	V	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.100	A	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.320	P	1.313	0.33	2.271	1.698	0.083	2.38	4.75
1.000	O	3.0104	1.604	5.083	3.1797	1.8958	5.4818	7.33
3.200	R	2.750	1.943	4.938	3.141	2.031	5.900	7.67

[a]Three swine/calculation
[b]P.I.I. based on 16 CFR 1500.41 and Draize Technique.

3.5 Research Studies.

3.5.1 Dental Dam Tests.

Following completion of the pilot study, a research study was performed using dental dam as an absorbent layer to simulate skin. Direct and vapor contact studies were conducted using contaminated and isopropanol-rinsed alkyd and polyurethane painted steel plate. Initial procedures involved three doses of agent of 0.5, 2.0, and 10.0 mg. Agent was applied to groups of 8 each, alkyd and polyurethane painted steel plates that were allowed to age for 30 min inside of fume hoods with a sash flow of 150 lfpm +30. At 30 min, the area of agent spread as well as the condition of the agent droplet (dry, damp, or wet) was checked and recorded. After 30 min, all plates were rinsed

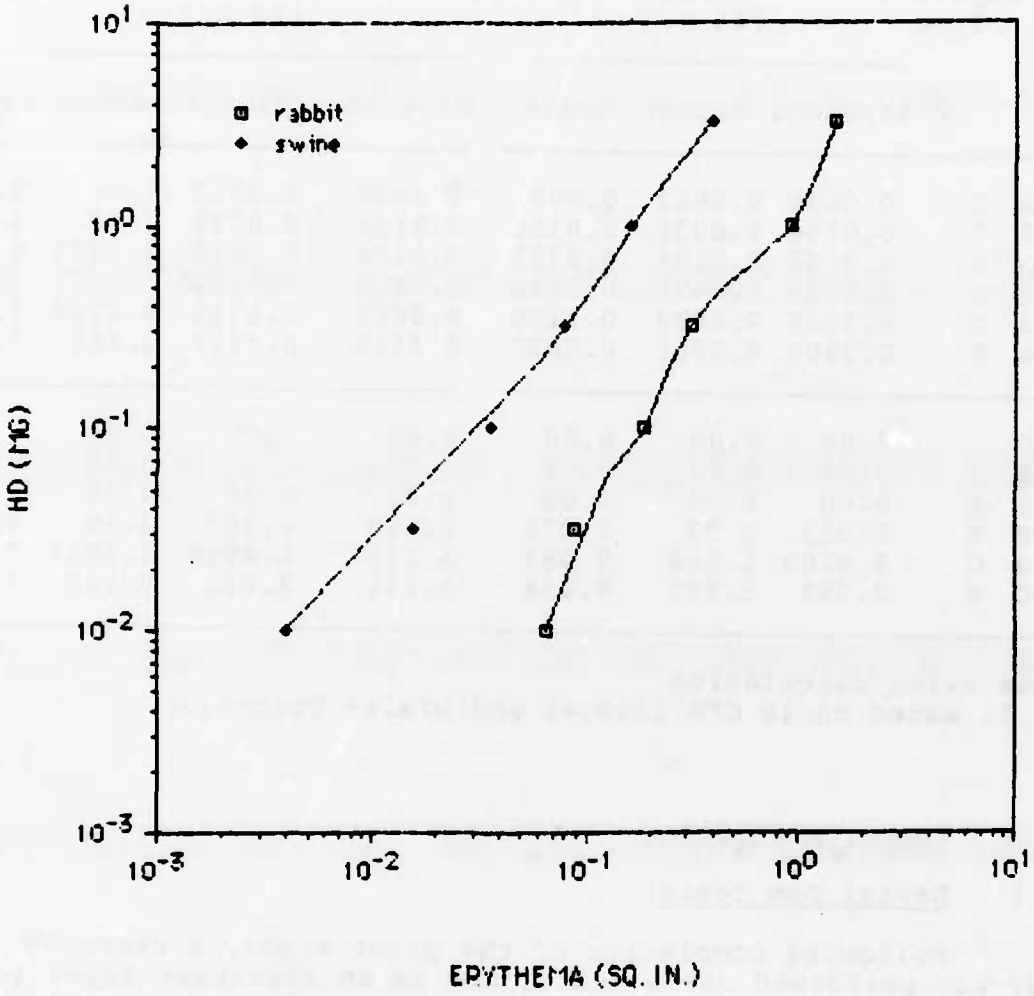

Figure. Skin Damaged Following 60-Min Direct Contact with HD, 24-Hr Erythema Response.

with isopropanol and then handled in one of three ways. Procedure I contacted the plates to dental dam for 60 min immediately after the rinse procedure. Procedure II involved an additional 15-min age after the isopropanol rinse before the 60-min contact. Procedure III involved an additional 5-hr age after rinsing and prior to the 60-min contact. In all tests, direct and vapor contact studies were conducted. A complete analysis of a series of control plates (groups of 8 for each dose level) as well as the dental dam and test plates was done chemically for monitoring either HD uptake (dental dam) or for residual HD content (steel plates).

Table 8. Rabbit/Swine Skin Irritation Ratios at 24 Hours Following 60-Minute Direct and Vapor HD Contact.

	24-Hr Erythema Response Ratio[a]	
HD Dose (mg)	Direct Contact	Vapor Contact
0.010	16.7	NR[b]
0.032	5.7	NR
0.100	5.3	NR
0.320	3.9	3.5
1.000	5.7	1.2
3.200	3.8	1.5

[a]Ratio of Rabbit/Swine response
[b]Swine did not respond

When conducting these tests, a standard rinse volume and procedure was used for removal of liquid HD from the contaminated steel plates. This procedure was also used in the animal tests described later. Table 9 lists the alcohol rinse volume in relation to the HD droplet size as well as the approximate dispersal time of the isopropanol. Following the 30-min age of the HD on the test plate, the plate was picked up with forceps and held in the mouth of a gallon jar containing 10% NAOH decontamination solution. The volume of isopropanol was then gently dispersed over the contamination, rinsing it directly into the decon solution. Solvent was dispersed through a stainless-steel, 16-gauge needle attached to a large syringe. Slight downward pressure was applied to the syringe plunger, and the solvent was applied in an up-down and side-side motion over the test plate.

Table 9. Procedure for HD Rinse-Removal from Painted Steel Plates.

Droplet Size (mg)	Solvent Volume (mL)	Approximate Dispersal Time (sec)
10.0	30.0	25.0
2.0	20.0	15.0
0.5	15.0	10.0

Following this rinse-removal process, tests were conducted with dental dam by the direct and vapor contact procedures.

3.5.1.1 Residual HD in Alkyd and Polyurethane Painted Steel Following a 30-Minute Age, Isopropanol Rinse, and 60-Minute Direct and Vapor Contact with Dental Dam.

During the previously described pilot study, definitive doses of liquid HD were tested on rabbit and swine skin by direct and vapor contact procedures to determine its effect on these two skin models. Responses of erythema and edema were measured and photographed for later comparison during the research phase of this project. The final task of this project was to determine the dose of HD contacting skin based on the measured skin response in rabbits. As an intermediate study, we substituted heavy dental dam for skin. Therefore, with a chemical analysis of the HD transferred to this medium, we would have an idea of how much HD could be transferred to rabbit skin under similar test conditions.

The first phase of this study was to evaluate the amount of HD transferred to dental dam following 60-min contact with alkyd and polyurethane painted steel plates that had dose levels of 0.5-, 2.0-, and 10.0-mg of neat HD applied to their surfaces. The contaminated plates were allowed to age for 30 min in fume hoods with a face velocity of 150 lfpm. After the 30-min age, the plates were rinsed with isopropanol (Table 9) and then wafted dry and immediately contacted to the dental dam. Direct contact plates were placed onto single thickness dental dam, contaminated side to the dam, and then taped to force contact. Vapor contact accomplished by placing a template on top of the dental dam and placing the contaminated plate into the template, forcing contamination towards the dam for 60 min. This unit was also sealed to prevent vapor escape. Following the 60-min contact, the steel plates and dental dams were placed into a recovery solvent of DEP with the volume of DEP dependent on the

starting dose of HD (Table 10). These DEP volumes were used throughout the dental dam and subsequent rabbit studies.

Table 10. DEP Volumes Used for HD Recovery/Analysis.

HD Dose (mg)	DEP Volume[a] (mL)
10.0	100.0
2.0	50.0
0.5	20.0[b]

[a]Steel plates placed into DEP volume as listed.
[b]All dental dam was placed into 20 mL of DEP regardless of dose of HD applied to the steel plate.

The results of the first tests are listed in Table 11 and show the mean and standard deviations for HD recovery from eight-group test plates and dental dams. For each dose level of HD, control plates, contact plates, and dental dams were evaluated. The first data to note is the column that depicts the spread of the HD on the two types of paint during the 30-min age.

The alkyd paint shows spreading (percentage area of a 1-in. by 2-in. plate) of 2.0, 0.5, and 0.25 for the 10.0-, 2.0-, and 0.5-mg doses of HD respectively. On the polyurethane plates, the HD spreads were 19.6-25.8%, 11.1-13.9%, and 3.4-5.6% for the three doses of HD. Polyurethane control plates retained more HD than alkyd plates at the 10.0- and 2.0-mg doses, but at the 0.5-mg dose, the alkyd plates retained more HD. This may be related to physicochemical factors of the surface that caused HD to dry or disappear on the polyurethane paint. In earlier studies,[1,2] polyurethane painted plates did not sorb and hold significant levels of HD or VX. There is obviously a significant difference in polyurethane paints and their resistance to the absorption of agent.

Table 11 data also indicates that when tested under the same conditions, the polyurethane paint can desorb its held HD more readily than does the alkyd paint. This effect could lead to more severe skin damage upon contact. It could also lead to a more rapid desorption of the contained HD if an extended age time was allowed after the initial decontamination-rinse removal of the surface contamination by either a solvent rinse or physical/chemical removal procedure.

Table 11. Residual HD in Alkyd and Polyurethane Painted Steel Following 30-Minute Age, Isopropanol Rinse, and 60-Minute Contact with Dental Dam.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate %	Condition[b] of Agent After 30" Age	ugs of HD Recovered/Chemical Analysis			
					Steel Plate		Dental Dam	
					Mean/S.D.	%/S.D.	Mean/S.D.	%/S.D.
	(mg)				306.0 ± 38.0	3.06 ± 0.38	----[c]	----[c]
		Control	2.0±0.0	Wet				
	10.0	Direct	2.0±0.0	Wet	135.0 ± 24.5	1.35 ± 0.25	105.3 ± 11.1	1.05 ± 0.11
A		Vapor	2.0±0.0	Wet	213.1 ± 25.4	2.13 ± 0.25	57.1 ± 10.4	0.57 ± 0.10
L								
K								
		Control	0.5±0.0	Wet	106.8 ± 11.5	5.34 ± 0.58	----	----
Y								
D	2.0	Direct	0.5±0.0	Wet	50.8 ± 9.1	2.54 ± 0.45	44.0 ± 7.15	2.20 ± 0.36
		Vapor	0.5±0.0	Wet	65.9 ± 14.9	3.30 ± 0.75	19.0 ± 2.4	0.95 ± 0.12
		Control	0.25±0.0	Wet	31.1 ± 2.1	6.22 0.42	----	----
	0.5	Direct	0.25±0.0	Wet	15.8 ± 3.8	3.15 ± 0.77	15.0 ± 3.5	2.99 ± 0.70
		Vapor	0.25±0.0	Wet	24.1 ± 3.5	4.81 ± 0.70	9.05 ± 3.58	1.81 ± 0.72

Table 11. Residual HD in Alkyd and Polyurethane Painted Steel Following 30-Minute Age, Isopropanol Rinse, and 60-Minute Contact with Dental Dam (Continued).

		Control	25.8 ± 6.7	Damp-Wet	416.0 ± 211.9	4.16 ± 2.12	----	----
POLYURETHANE	10.0	Direct	25.6 ± 5.0	Damp-Wet	25.3 ± 7.5	0.25 ± 0.08	462.0 ± 146.7	4.62 ± 1.47
		Vapor	19.6 ± 4.3	Damp-Wet	76.9 ± 23.6	0.77 ± 0.24	254.6 ± 58.8	2.55 ± 0.59
		Control	13.9 ± 2.9	Dry-Damp Wet	166.5 ± 44.9	8.33 ± 2.24	----	----
	2.0	Direct	12.0 ± 3.4	Dry-Damp Wet	15.6 ± 10.2	0.78 ± 0.51	164.5 ± 60.0	8.23 ± 3.00
		Vapor	11.1 ± 2.2	Dry-Damp Wet	31.1 ± 20.9	1.56 ± 1.04	102.1 ± 32.7	5.11 ± 1.64
		Control	3.4 ± 0.5	Dry	8.3 ± 1.8	1.66 ± 0.36	----	----
ANTHRANILE	0.5	Direct	5.6 ± 0.9	Dry	1.9 ± 0.4	0.38 ± 0.09	15.4 ± 2.7	3.08 ±0.55
		Vapor	5.0 ± 0.8	Dry	4.2 ± 0.8	0.84 ± 0.15	9.0 ± 4.0	1.80 ±0.80

[a]HD spread, mean area, and standard deviation of agent droplet size at 30 min after application. Test plates were 1-in. by 2-in. or 2.0 in.^{2}.
[b]Condition of agent from outer edge to center of droplet.
[c]Control plates not contacted to dental dam.

As the data indicate, more HD was transferred to dental dam by direct contact than by vapor contact, and the levels of HD transferred were high enough (ca. 10 µg or more) that they should cause an irritant response to rabbit skin. This would be verified in later tests that used rabbit skin rather then dental dam as the contacted surface.

The results of this test lead us to the second test series involving a second age of 15 min after the initial isopropranol rinse of the test plates.

3.5.1.2 Residual HD in Alkyd and Polyurethane Painted Steel Following a 30-Minute Age, Isopropanol Rinse, 15-Minute Additional Age, and 60-Minute Direct and Vapor Contact with Dental Dam.

This second set of tests involved procedures similar to those described in paragraph 3.5.1.1 with the only change being the additional 15-min age of the contaminated test plates after the isopropanol rinse. Detailed test results are shown in Table 12. The additional 15-min age at air flows of 150 lfpm substantially lowered the amount of HD contained in the control plates. For the alkyd plates, the retained HD was lowered by 22-38% (low-to-high dose); in the polyurethane plates, the residual HD content was lowered by as much as 50% in the low dose (0.5 mg) plates and 38% at the 2.0-mg dose. Forty-eight percent reduction of the retained HD was seen at the 10-mg dose. Similar effects were seen in the amounts of HD transferred to the dental dam. With the alkyd paint, the mean level of HD transferred by direct contact was more than that transferred by vapor. However, with the polyurethane painted plates, there was slightly more HD uptake by vapor contact than by direct contact. The results indicate that the alkyd and polyurethane plates at 0.5 mg should not be a serious hazard by either direct or vapor contact because a mean of only 2.0-8.7 µg of HD was transferred to dental dam. If ca. 10.0 µg of HD is the minimal effective dose for skin irritation in the rabbit, then we should see only minor skin damage when this test is repeated using the rabbit as the agent recipient.

Following this test, the third evaluation involved increasing the age time from 15 min to 5 hr following HD rinse removal from the painted steel plates.

Table 12. Residual HD in Alkyd and Polyurethane Painted Steel Following a 30-Minute Age, Isopropanol Rinse, 15-Minute Additional Age, and 60-Minute Contact with Dental Dam.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate	Condition[b] of Agent After 30" Age	µgs of HD Recovered/Chemical Analysis Steel Plate Mean/S.D.	Steel Plate %/S.D.	Dental Dam Mean/S.D.	Dental Dam %/S.D.
ALKYD	(mg)		%					
		Control	2.31 ±0.26	Wet	190.0 ±16.0	1.90 ±0.16	___[c]	___[c]
	10.0	Direct	2.19 ±0.26	Wet	141.3 ±10.9	1.41 ±0.11	56.8 ±5.2	0.57 ±0.05
		Vapor	2.19 ±0.26	Wet	160.0 ±13.1	1.60 ±0.13	39.8 ±2.6	0.40 ±0.03
	2.0	Control	0.5 ±0.0	Wet	65.9 ±8.7	3.30 ±0.43	—	—
		Direct	0.5 ±0.0	Wet	45.6 ± 7.2	2.29 ±0.36	20.5 ±2.9	1.03 ±0.15
		Vapor	0.5 ±0.0	Wet	57.5 ±11.1	2.88 ±0.55	13.8 ±0.7	0.69 ±0.04
	0.5	Control	0.25 ±0.0	Wet	24.3 ±3.5	4.24 ±1.57	—	—
		Direct	0.25 ±0.0	Wet	12.1 ±7.6	2.43 ±1.51	8.7 ±1.2	1.74 ±0.24
		Vapor	0.25 ±0.0	Wet	19.8 ±2.7	3.97 ±0.54	6.2 ±0.7	1.24 ±0.14
POLYURETHANE	10.0	Control	29.4 ±4.2	Damp-Wet	201.9 ±24.5	2.02 ±0.24	—	—
		Direct	25.9 ±7.2	Damp-Wet	24.4 ±5.0	0.24 ±0.05	112.8 ±49.2	1.13 ±0.49
		Vapor	27.5 ±3.8	Damp-Wet	35.6 ±14.5	0.36 ±0.15	116.3 ±33.6	1.16 ±0.34
	2.0	Control	13.0 ±2.5	Dry-Damp-Wet	63.4 ±24.4	3.18 ±1.22	—	—
		Direct	11.3 ±4.4	Dry-Damp-Wet	10.6 ±2.9	0.53 ±0.14	21.0 ±9.5	1.05 ±0.48
		Vapor	14.0 ±1.1	Dry-Damp	15.6 ±4.2	0.78 ±0.21	31.0 ±9.6	1.55 ±0.48
	0.5	Control	6.6 ±0.7	Dry	4.3 ±1.4	0.85 ±0.29	—	—
		Direct	5.6 ±1.1	Dry	3.6 ±0.7	0.72 ±0.14	2.0 ±0.9	0.40 ±0.18
		Vapor	5.6 ±2.5	Dry-Damp-Wet	4.8 ±2.9	0.97 ±0.58	5.3 ±5.8	1.05 ±1.16

[a]HD spread, mean area and standard deviation of agent droplet size at 30 min after application.
[b]Condition of agent from outer edge to center of deposit.
[c]Control plates not contacted to dental dam.

3.5.1.3 Residual HD in Alkyd and Polyurethane Painted Steel Following a 30-Minute Age, Isopropanol Rinse, 5-Hour Additional Age, and 60-Minute Direct and Vapor Contact with Dental Dam.

This study was the same as previous ones except that the age time of the rinsed plates was increased from 15 min to 5 hr. Table 13 lists the detailed analytical aspects of this phase. There is a reduction of the amount of residual HD in the steel plates when compared to that found in tests (Section 3.5.1.2). Tests show that the alkyd paint retained more HD at all three levels than the polyurethane painted plates retained. Of the amount of residual HD in the alkyd plates, only minimal HD was transferred to the dental dam (10 µg - high dose) by direct contact with lesser amounts transferred by vapor contact.

The polyurethane painted plates retained only minimal HD with 1.3-3.5 µg (mean) transferred to the dam. Based on past experience, this level of HD contamination should not be enough to cause observable irritation in rabbit skin.[1]

Based on the results of the three dental dam tests, it was decided that further work using rabbits as the biological model would produce valuable data to verify the dental dam studies. Therefore, three studies were conducted using procedures identical to those just described with rabbits substituted for dental dam.

3.5.2 Rabbit Studies.

Three skin irritation studies were conducted using previous procedures (Sections 3.5.1, 3.5.1.1, 3.5.1.2, and 3.5.1.3) but substituting rabbits as the monitor rather than dental dam. Damage to rabbit skin was evaluated, as during the pilot study (16 CFR 1500.41), with skin examined at 1 hr (at test-plate removal) and at 24, 48, and 72 hr. Photographs were taken of each rabbit that showed an observable skin irritation for up to 72 hr or for 24 hr after recovery if this occurred prior to 72 hr. Each skin irritation was measured to the nearest 1/8 in. (0.125 in.) for erythema, edema, and eschar or necrotic skin. For this report, only the 24-hr erythema observations were tabulated. However, the P.I.I. score was calculated from the scores for erythema and edema at 24 and 72 hr. Rabbits were tested in groups of eight with direct and vapor contact for each dose level and paint type. Along with concurrent controls, all painted steel test plates were analyzed chemically for residual HD content. Volumes of DEP used were the same as those listed in Table 10.

Table 13. Residual HD in Alkyd and Polyurethane Painted Steel Plates Following a 30-Minute Age, Isopropanol Rinse, 5-Hour Additional Age, and 60-Minute Contact with Dental Dam.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate	Condition[b] of Agent After 30" Age	µgs of HD Recovered/Chemical Analysis			
					Steel Plate		Dental Dam	
					Mean/S.D.	%/S.D.	Mean/S.D.	%/S.D.
ALKYD	(mg)	Control	% 2.06 +0.18	Wet	98.8 ± 8.8	0.99 ± 0.09	—[c]	—[c]
	10.0	Direct	2.25 +0.27	Wet	105.6 ±17.4	1.06 ± 0.17	10.0 ±0.7	0.010 ±0.007
		Vapor	2.13 +0.23	Wet	98.8 ±10.9	0.99 ±0.11	6.5 ±0.9	0.065 ±0.009
	2.0	Control	0.5 + 0.0	Wet	35.0 ± 6.3	1.75 ±0.31	—	—
		Direct	0.5 +0.0	Wet	35.9 ±4.4	1.80 ±0.22	4.8 ±0.7	0.24 ±0.04
		Vapor	0.5 +0.0	Wet	36.6 ±2.7	1.83 ±0.13	2.4 ±0.7	0.12 ±0.04
	0.5	Control	0.25 +0.0	Wet	8.2 ±2.7	1.63 ±0.54	—	—
		Direct	0.25 +0.0	Wet	10.0 ±0.9	2.00 ±0.18	1.6 ±0.3	0.32 ±0.06
		Vapor	0.25 +0.0	Wet	8.9 ±2.7	1.78 ±0.55	6.1 ±1.3	1.23 ±0.25
POLYURETHANE	10.0	Control	22.8 +2.5	Damp-Wet	16.3 ±3.5	0.16 ±0.03	—	—
		Direct	23.8 +8.7	Damp-Wet	17.5 ±7.0	0.18 ±0.07	3.5 ±0.8	0.035 ±0.08
		Vapor	30.0 +3.8	Damp-Wet	17.1 ±5.1	0.17 ±0.05	2.5 ±1.0	0.025 ±0.010
	2.0	Control	14.0 +2.3	Dry Damp-Wet	9.4 ±3.1	0.48 ±0.15	—	—
		Direct	14.0 +1.9	Dry-Damp Wet	9.5 ±0.9	0.48 ±0.04	2.2 ±1.1	0.11 ±0.06
		Vapor	13.8 +1.9	Dry-Damp Wet	10.8 ±2.3	0.55 ±0.12	2.1 ±0.21	0.10 ±0.01
	0.5	Control	5.5 +0.8	Dry	4.8 ±1.0	0.96 ±0.20	—	—
		Direct	4.8 +1.2	Dry	3.1 ±0.8	0.62 ±0.16	1.3 ±0.5	0.26 ±0.11
		Vapor	5.0 +0.8	Dry	5.5 ±1.2	1.10 ±0.23	1.7 ±0.4	0.35 ±0.08

[a]HD spread, mean area, and standard deviation of agent droplet size at 30 min after application.
[b]Condition of agent from outer edge to center of deposit.
[c]Control plates not contacted to dental dam.

3.5.2.1 Rabbit Skin Irritation Caused by Residual HD in Alkyd and Polyurethane Painted Steel Plates That Were Aged 30 Minutes, Isopropanol Rinsed, and Contacted to Skin for 60 Minutes by Direct and Vapor Methods.

The results of this study are listed in Table 14. As in the dental dam studies (Table 11), similar amounts of HD were retained in the painted steel plates. More HD was retained in the polyurethane paint than in the alkyd paint with the exception once more being the polyurethane plates at the 0.5-mg doses. These 0.5-mg plates had surface dried during the 30-min age; therefore, it appeared that less HD was retained in the poly than in the alkyd plates.

As mentioned earlier, the total spread of HD on the polyurethane plates was greater than the spread on the alkyd plates, contributing to the total retained HD as well as to the total area of skin damage in rabbits upon either direct or vapor contact. This was indeed what occurred during the 60-min rabbit contact conducted in this phase. For instance, by direct contact with the alkyd plates, the degree (P.I.I. score) of skin irritation ranged from 6.87 to 7.82 (low-to-high dose), whereas the area for erythema ranged from a mean of 0.21 to 1.06 $in.^2$. These P.I.I. scores indicate that the intensity of the damage varied little with the total amount of HD coming in contact with the skin. However, the mean surface area of the damage was considerably different. The higher the HD dose (in micrograms) the larger the surface area involved in the injury. This also held true for other related irritation aspects of the injury, including edema and eschar formation.

As shown in Table 14, the intensity of the skin damage by vapor contact was less than direct contact, especially from the contaminated alkyd painted steel plates. The size of the damage was larger only at the starting dose of 10.0 mg. We would expect the vapor damage area to be larger than the direct contact damage area but somewhat less intense (severe). This was proven during the polyurethane test portion of this study. Note the larger damage areas 2+ to 3+ in. at the 10.0-mg starting dose as well as the general intensity of the damage (P.I.I. of 8.0 at 10 mg). At the 2.0-mg dose, the damaged area from HD-contaminated polyurethane was over 2.0 in. compared to 0.49 or less inches from the HD-contaminated alkyd plates. Only the 0.5-mg plates showed no effect with alkyd plates; however, we still saw minimal effects with the vapor phase of polyurethane plates at this dose level. This was because 1/8 rabbits showed some skin irritation.

The data in Table 14 support the concept that larger areas of skin damage associated with both vapor and polyurethane test plates is due first to the spread of the vapor within the sealed template and then to the total spread of the HD over the surface of the polyurethane paint. Vapor from HD in our test

Table 14. Residual HD in Alkyd and Polyurethane Painted Steel Plates Following a 30-Minute Age, Isopropanol Rinse, 60-Minute Contact with Rabbit Skin; Skin Damage Evaluation.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate	Condition[b] of Agent After 30" Age	µgs of HD Recovered Chemically — Steel Plate		Rabbit Skin Irritation	
					Mean/S.D.	%/S.D.	P.I.I.[c] Score	24 hrd Erythema Mean & S.D.
	(mg)		%					(sq. in.)
ALKYD	10.0	Control	2.0 ± 0.0	Wet	322.5 ± 16.5	3.23 ±0.16	—[e]	—[e]
		Direct	2.0 ± 0.0	Wet	233.8 ± 25.2	2.34 ±0.25	7.82	1.06 ±0.45
		Vapor	2.0 ± 0.0	Wet	206.3 ± 38.7	2.06 ±0.39	4.13	1.31 ±0.81
	2.0	Control	0.5 ± 0.0	Wet	91.0 ± 6.4	4.55 ±0.32	—	—
		Direct	0.5 ± 0.0	Wet	75.6 ± 6.8	3.78 ±0.34	7.63	0.49 ±0.21
		Vapor	0.5 ± 0.0	Wet	61.3 ± 7.7	3.07 ±0.39	0.75	0.14 ±0.20
	0.5	Control	0.25 ± 0.00	Wet	29.8 ± 3.7	5.96 ±0.93	—	—
		Direct	0.25 ± 0.00	Wet	18.6 ± 3.7	3.73 ±0.75	6.87	0.21 ±0.13
		Vapor	0.25 ± 0.0	Wet	23.7 ± 4.7	4.74 ±0.93	0.00	0.00 ±0.00
POLYURETHANE	10.0	Control	19.8 ± 2.8	Damp-Wet	418.1 ±127.3	4.18 ±1.27	—	—
		Direct	22.0 ± 6.9	Damp-Wet	17.0 ± 8.6	0.17 ±0.09	8.00	2.67 ±1.42
		Vapor	26.3 ± 9.5	Damp-Wet	25.8 ± 17.3	0.26 ±0.17	7.57	3.92 ±1.45
	2.0	Control	11.9 ± 1.7	Dry-Damp-Wet	114.0 ± 31.0	5.70 ±1.55	—	—
		Direct	10.4 ± 2.2	Dry-Damp	6.9 ± 6.2	0.35 ±0.31	7.82	2.06 ±0.75
		Vapor	12.4 ± 1.5	Dry-Damp	4.3 ± 2.1	0.21 ±0.11	5.85	2.55 ±1.15
	0.5	Control	4.5 ± 0.8	Dry	18.4 ± 8.3	3.69 ±1.65	—	—
		Direct	3.6 ± 0.8	Dry	1.7 ± 0.4	0.33 ±0.09	6.70	0.27 ±0.17
		Vapor	3.6 ± 0.4	Dry	2.6 ± 0.7	0.52 ±0.14	0.06	0.09 ±0.27

[a]HD spread, mean area and standard deviation of agent droplet size at 30 min after application (8 plates each).
[b]Condition of agent from outer edge to center of droplet.
[c]Primary Irritation Index score - 16 CFR 1500.41.
[d]Square inch area for erythema at 24-hr evaluation (8 animals).
[e]Control plates not contacted with rabbit skin.

procedure usually resulted in a large skin damage area; however, this damage was generally somewhat reduced in severity.

Based on the results of this study, we decided to go on to the next phase and repeat the test following an additional 15-min age of the test plates following the 30-min age and isopropanol rinse procedure.

3.5.2.2 Rabbit Skin Irritation Caused by Residual HD in Alkyd and Polyurethane Painted Steel Plates That Were Aged 30 Minutes, Isopropanol Rinsed, Aged an Additional 15 Minutes, and Contacted to Skin for 60 Minutes by Direct and Vapor Methods.

This test was a duplicate study of the one performed in Section 3.5.1.2 with rabbits used as the test monitor rather than dental dam. Detailed analytical data are summarized in Table 15. Once again, as noted in Table 12, the data show that the additional 15-min age after the isopropanol rinse significantly reduced the HD contamination in all test plates, resulting in a reduction in the intensity and size of skin damage in the rabbits. Note that skin damage did not occur in the 16 rabbits exposed by vapor to either the alkyd or polyurethane test plates at the 0.5-mg dose. It is also evident that all vapor doses caused a reduced P.I.I. score as well as a significant reduction in the size of the skin injury when compared to data in Table 14.

Based on these results, the final test planned was to extend the age after rinsing with isopropanol to 5 hr and then contact the plates to rabbit skin for 60 min by the direct and vapor procedures.

3.5.2.3 Rabbit Skin Irritation Caused by Residual HD in Alkyd and Polyurethane Painted Steel Plates That Were Aged 30 Minutes, Isopropanol Rinsed, Aged an Additional 5 Hours, and Contacted to Skin for 60 Minutes by Direct and Vapor Methods.

This final test used rabbits and involved a 5-hr age of the painted test plates following the isopropanol rinse prior to the 60-min contact with rabbit skin. Data are shown in Table 16, and it is noted immediately that no skin injury was observed in any rabbits following 60-min vapor contact for either the alkyd or polyurethane painted test plates. The levels of contamination in the alkyd plates at the 10.0- and 2.0-mg starting doses should have been enough to cause some visible damage; however, referring back to Table 13, it is noted that at these conditions, only 6.0-10.0 µg of HD were transferred to dental dam under the same test conditions. Therefore, the amount of HD vapor is not enough to elicit a visible skin injury to rabbit skin. The same levels emitted during direct contact did produce observable damage in 7/8 rabbits at the 0.5-mg dose

Table 15. Residual HD in Alkyd and Polyurethane Painted Steel Plates Following a 30-Minute Age, Isopropanol Rinse, Additional 15-Minute Age, and 60-Minute Contact with Rabbit Skin; Skin Damage Evaluation.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate	Condition[b] of Agent After 30" Age	µgs of HD Recovered Chemically Steel Plate		Rabbit Skin Irritation	
					Mean/S.D.	%/S.D.	P.I.I.[c] Score	24 hr[d] Erythema Mean & S.D.
	(mg)		%					(sq. in.)
ALKYD	10.0	Control	2.0 ± 0.0	Wet	176.9 ± 11.0	1.77 ± 0.11	—[e]	—[e]
		Direct	2.0 ± 0.0	Wet	138.8 14.8	1.39 ± 0.15	7.63	0.64 ± 0.33
		Vapor	2.25 ± 0.27	Wet	165.0 ± 24.0	1.65 ± 0.24	3.23	1.00 ± 0.30
	2.0	Control	0.5 ± 0.0	Wet	55.0 ± 5.4	2.75 ± 0.27	—	—
		Direct	0.5 ± 0.0	Wet	53.8 ± 9.3	2.69 ± 0.46	7.63	0.38 ± 0.12
		Vapor	0.5 ± 0.0	Wet	50.3 ± 4.1	2.52 ± 0.21	0.06	0.03 ± 0.09
	0.5	Control	0.25 ± 0.00	Wet	17.6 ± 4.4	3.52 ± 0.87	—	—
		Direct	0.25 ± 0.00	Wet	15.8 ± 3.4	3.15 ± 0.68	6.38	0.12 ± 0.06
		Vapor	0.25 ± 0.00	Wet	18.8 ± 3.1	3.77 ± 0.62	0.00	0.00 ± 0.00
POLYURETHANE	10.0	Control	27.0 ± 4.0	Damp-Wet	208.1 ± 68.4	2.08 ± 0.68	—	—
		Direct	21.3 ±30.0	Damp-Wet	12.0 ± 2.9	0.12 ± 0.03	8.00	2.21 ± 1.07
		Vapor	3.8 ±12.6	Damp-Wet	8.6 ± 2.3	0.09 ± 0.02	7.04	2.92 ± 0.58
	2.0	Control	13.5 ± 1.2	Damp	93.4 ± 45.6	4.67 ± 2.28	—	—
		Direct	12.1 ± 3.0	Damp	4.1 ± 1.6	0.21 ± 0.08	8.00	1.17 ± 0.29
		Vapor	4.0 ± 0.8	Dry-Damp-Wet	4.4 ± 0.6	0.22 ± 0.03	2.76	1.06 ± 0.97
	0.5	Control	4.0 ± 0.8	Dry	5.6 ± 1.6	1.12 ± 0.33	—	—
		Direct	3.8 ± 0.9	Dry	1.6 ± 0.5	0.33 ± 0.10	3.73	0.16 ± 0.12
		Vapor	4.4 ± 1.1	Dry	1.6 ± 0.4	0.32 ± 0.08	0.00	0.00 ± 0.00

[a]HD spread, mean area and standard deviation of agent droplet size at 30 min after application (8 plates each).
[b]Condition of agent from outer edge to center of droplet.
[c]Primary Irritation Index score - 16 CFR 1500.41.
[d]Square inch area for erythema at 24-hr evaluation (8 animals).
[e]Control plates not contacted with rabbit skin.

Table 16. Residual HD in Alkyd and Polyurethane Painted Steel Plates Following a 30-Minute Age, Isopropanol Rinse, Additional 5-Hour Age, and 60-Minute Contact with Rabbit Skin; Skin Damage Evaluation.

Paint Type	HD Dose	Contact Type	HD[a] Spread on Plate	Condition[b] of Agent After 30" Age	µgs of HD Recovered Chemically Steel Plate		Rabbit Skin Irritation	
					Mean/S.D.	%/S.D.	P.I.I.[c] Score	24 hrd Erythema Mean & S.D.
	(mg)		%					(sq. in.)
ALKYD		Control	2.0 ± 0.0	Wet	90.0 ± 10.0	0.90 ± 0.10	—[e]	—[e]
	10.0	Direct	2.0 ± 0.0	Wet	80.0 ± 7.1	0.80 ± 0.71	7.51	0.28 ± 0.09
		Vapor	2.0 ± 0.0	Wet	76.3 ± 7.9	0.76 ± 0.08	0.00	0.00 ± 0.00
	2.0	Control	0.5 ± 0.0	Wet	31.6 ± 6.4	1.58 ± 0.32	—	—
		Direct	0.5 ± 0.0	Wet	11.4 ± 2.2	0.58 ± 0.11	5.51	0.06 ± 0.03
		Vapor	0.5 ± 0.0	Wet	10.5 ± 1.5	0.53 ± 0.08	0.00	0.00 ± 0.00
	0.5	Control	0.25 ± 0.00	Wet	11.3 ± 2.3	2.25 ± 0.46	—	—
		Direct	0.25 ± 0.00	Wet	9.4 ± 2.2	1.88 ± 0.44	4.07	0.026 ± 0.015
		Vapor	0.25 ± 0.00	Wet	10.0 ± 0.5	2.00 ± 0.11	0.00	0.00 ± 0.00
POLYURETHANE	10.0	Control	22.3 ± 6.8	Damp-Wet	13.7 ± 4.4	0.14 ± 0.04	—	—
		Direct	20.6 ± 5.3	Damp-Wet	9.5 ± 1.4	0.095 ± 0.014	1.91	0.30 ± 0.28
		Vapor	21.5 ± 4.5	Damp-Wet	10.1 ± 1.8	0.10 ± 0.02	0.00	0.00 ± 0.00
	2.0	Control	13.9 ± 1.3	Dry-Damp	7.7 ± 2.0	0.37 ± 0.09	—	—
		Direct	13.8 ± 1.3	Dry-Damp	4.4 ± 0.6	0.23 ± 0.03	1.69	0.18 ± 0.18
		Vapor	10.4 ± 4.1	Dry-Damp-Wet	5.4 ± 0.9	0.27 ± 0.05	0.00	0.00 ± 0.00
	0.5	Control	4.1 ± 0.9	Dry	2.1 ± 0.4	0.42 ± 0.07	—	—
		Direct	5.0 ± 0.8	Dry	1.4 ± 0.4	0.28 ± 0.08	0.75	0.018 ± 0.05
		Vapor	4.0 ± 1.1	Dry	3.5 ± 0.6	0.70 ± 0.13	0.00	0.00 ± 0.00

[a]HD spread, mean area and standard deviation of agent droplet size at 30 min after application (8 plates each).
[b]Condition of agent from outer edge to center of droplet.
[c]Primary Irritation Index score - 16 CFR 1500.41.
[d]Square inch area for erythema at 24-hr evaluation (8 animals).
[e]Control plates not contacted with rabbit skin.

and in 8/8 rabbits at the 2.0- and 10.0-mg starting doses. The area of the damage was again reduced in size from that observed in the previous test, but the intensity of the damage was still high (P.I.I. score of 4.07-7.51).

During the polyurethane direct contact phase, only 1/8 rabbits at the 0.5-mg starting dose showed a visible skin irritation, and at the 2.0- and 10.0-mg starting doses, 6/8 rabbits showed visible and measurable skin irritation. These injuries were significantly reduced in size and intensity from those seen in the previous test (Table 15). These results indicated that HD contamination is more readily removed from this particular polyurethane paint than from this alkyd paint either by additional aeration or by physical or chemical means. However, the data also show that HD trapped in alkyd paint may be reduced with aging to levels that will not be a significant hazard by vapor contact and can also be reduced significantly in total hazard with proper aeration and physical/chemical removal so that direct contact injuries are much reduced.

4. DISCUSSION AND CONCLUSION

This experiment was designed to determine if a measured amount of HD contamination would produce repeatable and measurable skin damage in rabbits and also if measurable skin damage in rabbits could be used to determine the contamination level of HD being transferred to the skin from contaminated painted steel plates.

In conjunction with this test design, we first conducted a controlled study (Phase II) exposing rabbits to precise microgram and milligram amounts of neat liquid HD by direct and vapor contact procedures. These agent doses were established from 0.010 to 3.2 mg and were spaced at 0.5-log intervals (because it was believed that skin would not respond to closer log doses) with an observable difference in the total damage area (square inches). Rabbits in groups of three were tested, and we did note measurable differences in the size of the skin irritation using the mean response of each dose group based on the 24-hr erythemic area. However, because of skin differences (skin thickness, hair growth patterns, etc.) among rabbits, we also observed a range of individual responses to the same levels of HD contamination. The skin irritation data from this rabbit study indicates that this species is sensitive to HD to such a degree that its skin response may require HD-dose separation at levels greater than 0.5-log interval and may approach the 1.0- to 1.5-log interval for statistically significant differentiation in erythema response. HD dosed by the vapor route by our procedure shows an even greater disparity in its ability to produce an erythema response that can be related to a definite HD-log dose. A statistical evaluation of the 24-hr erythema response in rabbits is shown in Table 17.

Table 17. Statistical Summary of 24-Hour Erythema Responses in Rabbits From 0.5-Log Interval Doses of Percutaneous HD.

Dose Group	HD Dose (mg)	Exposure Type	24 Hr Erythema (sq. in)			F. Distribution/Analysis of Variance* Group No.					
			Mean	S.D.	Range	1	2	3	4	5	6
1	0.010	D	0.065	±0.032	0.035-0.098	---	0.56	2.72	184.69	4.46	30.35
2	0.032	I	0.089	±0.045	0.063-0.141	0.56	----	1.67	74.22	4.21	29.19
3	0.100	R	0.188	±0.125	0.063-0.313	2.72	1.67	----	3.00	3.18	23.57
4	0.320	E	0.313	±0.00	0.313-0.313	184.69	74.22	3.00	----	2.26	20.75
5	1.000	C	0.922	±0.702	0.141-1.500	4.46	4.21	3.18	2.26	---	1.35
6	3.200	T	1.479	±0.444	1.00-1.875	30.35	29.19	23.57	20.75	1.345	----
1	0.010	V	0.000	±0.000	0.000-0.00	----	1.00	62.95	61.59	119.71	32.29
2	0.032	A	0.467	±0.72	0.000-1.25	1.00	----	22.82	33.87	36.94	20.05
3	0.100	P	3.177	±0.69	2.406-3.75	62.95	22.82	----	4.04	0.88	0.60
4	0.320	O	4.604	±1.016	3.50 - 5.50	61.59	33.87	4.04	- - - -	1.93	0.14
5	1.000	R	3.67	±0.58	3.00 - 4.06	119.71	36.94	0.88	1.93	----	0.51
6	3.200		4.25	±1.30	3.06-5.63	32.29	20.05	1.60	0.14	0.51	----

*Significantly different at 95% confidence level if F value is >7.71.

Based on the mean and standard deviation among the six dose levels tested, there is a difference in their ability to cause erythema; however, we can see by the range of these effects that a great deal of individual variation (biological variation) produces response overlaps at all six dose levels. An analysis of variance is part of Table 17, and all values in the table with an F distribution greater than 7.71 are significantly different at the $p = 0.05$ level. We see that dose levels of 0.010, 0.032, and 0.100 mg by direct contact are not statistically different even though differences were evident in mean measured responses. The dose of 1.0 mg (group 5) is not statistically different from any other group because of the minimal response of one rabbit in the group. Group 6 is different from groups 1-4 but not from group 5. By the vapor route, groups 1 and 2 were similar (basically no response), and groups 3-6 were similar. This similarity occurs because the 1-in. by 2-in. template, as stated earlier, forms a chamber that allows the HD vapor to quickly reach equilibrium and causes an effect over the total area of skin under the template. This is noted in the mean area of response as well as in the F distribution values for these four groups.

As part of this study, a preliminary test was conducted using swine in groups of three with the same dose levels of HD used on the rabbits. Table 18 lists the 24-hr erythema responses resulting from these exposures, and we see that responses among the six dose levels do not overlap in the swine as noted in the rabbit. Swine had very uniform skin but rabbits did not. In addition, there was little or no measurable difference in the response elicited in each swine in the groups at dose levels of 0.010 to 0.10 mg/kg by direct control. As shown in Table 18, the irritation produced by 0.010 mg of HD was significantly different from all other doses used at the $p = 0.05$ level. This was also true for the 0.032-mg dose. The statistical analysis of the swine direct and vapor contact data using analysis of variance where the F distribution value of 7.71 is significant at the $p = 0.05$ show these data to be less significant as the dose levels approach 3.2 mg. However, upon reanalysis of the data using the Bonferroni test,[14] the data with the asterisks in Table 18 was significant at the 0.05 level.

In Phase III of the study, rabbits were exposed to painted steel alkyd or polyurethane test plates after the plates were contaminated with either 10.0, 2.0, or 0.5 mg of neat HD. Following a 30-min age and alcohol rinse, plates were contacted to rabbit skin for 60 min. A duplicate series of tests substituted dental dam for rabbit skin in an attempt to measure the amount of HD actually transferred to the rabbit. There was good agreement among the test plates and the amount of HD left on them after contacting dental dam or rabbit skin. The amount of HD transferred to the dam and recovered by chemical analysis is assumed to be near the amount transferred to rabbit skin.

Table 18. Statistical Summary of 24-Hour Erythema Responses in Swine From 0.5-Log Interval Doses of Percutaneous HD.

Dose Group	HD Dose (mg)	Exposure Type	24 Hr Erythema (sq. in) Mean	S.D.	Range	F. Distribution/Analysis of Variance* Group No. 1	2	3	4	5	6
1	0.010	D	0.0039	±0.000	0.0039-0.0039	----	100.0	100.0	54.5	25.0	8.4
2	0.032	I	0.0156	±0.000	0.0156-0.0156	100.0	----	100.0	38.9	21.4	7.9
3	0.100	R	0.0353	±0.000	0.0353-0.0353	100.0	100.0	----	18.6	16.0	7.1**
4	0.320	E	0.0796	±0.0178	0.0625 0.0980	54.5	38.9	18.6	---	6.1	5.4**
5	1.000	C	0.1615	±0.0549	0.110-0.219	25.0	21.4	16.0	6.1	---	2.8
6	3.200	T	0.391	±0.231	0.234-0.656	8.4	7.9	7.1**	5.4**	2.8	----
1	0.010	V	0.0000	±0.000	0.000	----	----	----	7.2**	100.0	100.0
2	0.032	A	0.0000	±0.000	0.000	----	----	----	7.2**	100.0	100.0
3	0.100	P	0.0000	±0.000	0.000	----	----	----	7.2**	100.0	100.0
4	0.320	O	1.313	±0.846	0.500-2.188	7.2**	7.2**	7.2**	----	9.1	7.6**
5	1.000	R	3.010	±0.485	2.656-3.563	100.0	100.0	100.0	9.1	----	0.6
6	3.200		2.750	±0.308	2.50 - 3.09	100.0	100.0	100.0	7.6**	0.6	----

*Significantly different at 95% confidence level if F value is >7.71.

**Significantly different at p = 0.05 level using the Bonferroni[14] method of analysis.

Table 19 is a compilation of the amount of HD transferred to dental dam during a 60-min contact. At the 10.0- and 2.0-mg doses, alkyd painted steel released less agent to the dam than polyurethane painted steel did. This could be a transport problem; however, it is probably related to initial spread of the agent being much greater on the polyurethane paint. With a greater agent spread, there was more agent surface to dam contact and therefore more agent could be transferred during the time of contact. Whether or not a correlation can be made between dose of agent transferred and response in rabbit skin or vice versa, responses in rabbit skin used to predict amount of HD transferred was one of the reasons for conducting these tests. In Table 19, we see that for alkyd paint, 105.3 µg of HD was transferred to dental dam after 60-min of direct contact. The agent produced a mean erythema of 1.06 $in.^2$ in eight rabbits. In Table 17, by direct contact, 100 µg of HD produced a mean erythema of 0.188 $in.^2$. This seems to be a rather large discrepancy in size of erythema produced. However, we must take into account that the data from Table 17 were produced by a minute drop of neat agent under direct contact. The data in Table 19 were developed from a larger drop of agent that had spread over the test plate for 30 min then was alcohol rinsed. The end result was to spread the trapped agent over a larger area, thereby producing the enlarged skin response.

The problem in measuring skin damage, as related to agent dose, will continue unless a confined equal size agent source is used during a skin test procedure. This measurement problem is more apparent when the data from the polyurethane paint study is examined. The spread of agent on the polyurethane paint was greater than the spread on the alkyd paint and therefore had a greater reservoir area from which to produce injury. For example, in Table 19, a dose of 462.0 µg of HD was transferred to rabbit skin and produced an average erythema of 2.67 $in.^2$. In Table 17, a controlled dose of 0.462 mg of neat HD by direct contact would be expected to produce an erythema response of about 0.400 $in.^2$. In actuality, the 462 µg of HD contained in polyurethane paint produced a wound six times larger than that, probably due to the spread of the agent over the painted surface of the steel plate. From these test data, it is evident that a controlled agent droplet can be expected to produce a wound that can be related to the dose of agent. But, a response in skin can only be used to interpret the dose of agent that caused it if that dose of agent is confined to a standardized area. Once agent spreads and contaminates a surface, removal of the contamination does not remove the trapped residue. Because it has spread, the residue will inflict a much larger wound than would a single discrete droplet of the same milligram weight.

Table 19. Rabbit Skin Irritation From Suspected Levels of HD Transferred From Painted Steel Plates By Direct or Vapor Contact, As Related to HD Levels Recovered From Dental Dam Under the Same Test Conditions.

Steel Plate Paint Type	Starting HD Dose (mg)	Contact Type	Age Time After Alcohol (min)[a]	µgs of HD[b] Recovered Dental Dam	RABBIT SKIN IRRITATION Erythema (24 Hr) Mean (sq inches)	P.I.I. Score[c]
	10.0	Direct	0.0	105.3	1.06	7.82
			15.0	56.8	0.64	7.63
			300.0	10.0	0.28	7.51
A		Vapor	0.0	57.1	1.31	4.13
			15.0	39.8	1.00	3.23
L			300.0	6.5	0.00	0.00
	2.0	Direct	0.0	44.0	0.49	7.63
K			15.0	20.5	0.38	7.63
			300.0	4.8	0.06	5.51
Y		Vapor	0.0	19.0	0.14	0.75
			15.0	13.8	0.03	0.06
D			300.0	2.4	0.00	0.00
	0.5	Direct	0.0	15.0	0.21	6.87
			15.0	8.7	0.12	6.38
			300.0	1.6	0.03	4.07
		Vapor	0.0	9.1	0.00	0.00
			15.0	6.2	0.00	0.00
			300.0	6.1	0.00	0.00
	10.0	Direct	0.0	462.0	2.67	8.00
			15.0	112.8	2.21	8.00
			300.0	3.5	0.30	1.91
		Vapor	0.0	254.6	3.92	7.57
			15.0	116.3	2.92	7.04
P			300.0	2.3	0.00	0.00
O	2.0	Direct	0.0	164.5	2.06	7.82
L			15.0	21.0	1.17	8.00
Y			300.0	2.2	0.18	1.69
U		Vapor	0.0	102.1	2.55	5.85
R			15.0	31.0	1.06	2.76
E			300.0	2.1	0.00	0.00
T						
H	0.5	Direct	0.0	15.4	0.27	6.70
A			15.0	2.0	0.16	3.73
N			300.0	1.3	0.18	0.75
E		Vapor	0.0	9.0	0.09	0.06
			15.0	5.3	0.00	0.00
			300.0	1.7	0.00	0.00

[a]HD on steel plates for 30 min prior to alcohol rinse.
[b]Mean value of HD from eight plates.
[c]Primary Irritation Index Score - 16 CFR 1500.41.

The following conclusions can be derived from the data:

- Rabbit skin is extremely sensitive to HD, and as little as 0.010 mg or less will produce an observable skin irritation after 60 min of direct contact.

- Swine skin is less reactive to HD than rabbit skin; however, the response produced on swine skin following HD contact is much more uniform among different swine than the irritation response produced in rabbit skin.

- Preliminary delivery tests done with dental dam indicates this material to be a very good substitute for skin; also, it provided valuable insight into the levels of HD transferred to rabbit skin by direct and vapor contact.

- A known amount of HD, when applied directly to either rabbit or swine skin, produced a response that was definable at about 0.5-log interval. However, when fluxing from a painted surface, similar levels of HD contamination produced much larger areas of skin damage. The damage was larger, and the size of the damage was related to spread of the agent over the painted surface.

- When applied to these particular alkyd and polyurethane painted steel plates, equal amounts of HD do not produce the same effect on skin. Because agent tended to penetrate and spread more on the polyurethane paint, the damage produced in rabbit skin is larger from the contaminated polyurethane paint than from the contaminated alkyd paint.

- Trying to interpret agent dose for an elicited skin response in rabbits is possible but only within the limits of about 1.0- to 1.5-log intervals. This is the case because contamination under painted surfaces produces a much larger irritation than does the same amount of HD if deposited as a single droplet in a confined area.

Blank

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